

Polyphasic characterization of microbial communities under the stressful conditions of nitrate, heavy metals, radionuclides, and acidic pH in contaminated groundwater

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Clonal libraries of multiple genes (SSU rRNA gene, *nirK*, *nirS*, *amoA*, *pmoA*, and *dsrAB*; n=6100) were constructed from groundwater samples (n=6) that varied in degrees of contamination. The sites were in proximity to a concentration plume, and the following geochemical parameters were measured: pH, nitrate, uranium, nickel, sulfate, and TOC (total organic carbon). The *nir* genes encode nitrite reductase, *amo/pmoA* encode ammonia monooxygenase, and *dsrAB* encode sulfite reductase. Principle components analysis (PCA) was used to compare the relationships between the sites with respect to the unique operational taxonomic unit (OTU) distributions within the different clonal libraries. When geochemical characteristics were analyzed with PCA, the data suggested that the samples varied significantly with respect to nitrate and sulfate. The sites were grouped differently when compared upon the basis of the SSU rDNA genes or each of the functional genes. The diversity of *amoA* genes was much lower compared to the other functional genes, and the one sample with high nitrate and circum neutral pH appeared to be different from the other sites. When all gene OTUs were used in the analyses, the sites were more similar than in any other comparison, and the background site was grouped with the acidic, contaminated sites. The results indicated that a combination of the contaminants (i.e., nitrate, uranium, nickel) and pH levels have impacted the bacterial communities at the respective sites in different and dynamic ways. These data suggest that even though the background site was phylogenetically distinct from the acidic sites, that the extreme conditions of the acidic samples might be more analogous to the limiting nutrient conditions of the background site.